

AMENDMENTS TO THE SPECIFICATION

Please amend the specification, without prejudice, by substituting the following paragraphs:

Please amend the paragraph starting at page 2, line 22, as follows:

It was known that the proliferation and differentiation of the stem cells would be effected by the growth factors, such as ~~EGF, GM-CSF~~ epidermal growth factor (EGF), granulocyte-monocyte colony stimulating factor (GM-CSF), etc. When the growth factors in the culture environment are changed, the stem cells differentiate into different cells along with the specificity of the growth factors. For example, GM-CSF can act on a specific receptor complex present on hematopoietic progenitor cells, and thus, can promote the proliferation and differentiation of the hematopoietic progenitor cells in the bone marrow into monocyte, neutrophil, etc. Therefore, GM-CSF may be used to treat the diseases related to leukocyte deficiency.

Please amend the paragraph starting at page 7, line 24, as follows:

The methanol-extracted product according to this invention has been analyzed to have a reverse-phase ~~HPLC~~ High Performance Liquid Chromatography (HPLC) elution profile as shown in Figure 1.

Please amend the paragraph starting at page 9, line 7, as follows:

Figure 1 shows the reverse-phase HPLC elution profiles of ~~PoMuM~~ the methanol-extracted product of Polygonum multiflorum Thunb. (PoMuM) detected at three different wavelengths, in which ~~red~~ the middle line (20): 312 nm, ~~blue~~ the upper line (10): 254 nm, ~~green~~

the lower line (30): 280 nm; and in which the units of the x axis is "time (minutes)" and that of the y axis is "intensity (norm.);"

Please amend the paragraph starting at page 9, line 10, as follows:

Figure 2 shows the survival rates of dimethylnitrosamine (DMN)-treated mice, in which the mice were orally administered with 0 mg/kg (●), 100 mg/kg (○), 200 mg/kg (▼) and 40 mg/kg (▽) of PoMyM (n=4 for each group) and survival of mice was monitored daily;

Please amend the paragraph starting at page 9, line 14, as follows:

Figures 3 and 4 respectively show the gross views and the histopathological examination results of livers taken from three different mice, in which panel A: the liver of a mouse receiving PBS Phosphate Buffered Saline (PBS) intraperitoneally for 12 days (control group); panel B: the liver of a mouse which received the DMN treatment as described in Example 3 for 12 days; and panel C: the liver of a mouse which received the DMN treatment as described in Example 3 for 12 days, followed by oral administration of 40 mg/kg PoMuM for 28 days.

Please amend the paragraph starting at page 9, line 22, as follows:

Figure 5 shows the cell proliferative effect of ~~PoMuMPh~~ n-hexane-extracted product from the methanol-extracted product of Polygonum multiflorum Thunb. (PoMuMPh) upon the primary culture of bone marrow cells established from 4-6 week-old mice, in which panel A: vehicle; panel B: 1µg/ml of PoMuMPh; panel C: 10 µg/ml of PoMuMPh; and panel D: 100 µg/ml of PoMuMPh (magnification 100X);

Please amend the paragraph starting at page 10, line 9, as follows:

Figure 8 shows the effect of PoMuM in promoting the recovery of leukocytes in peripheral bloods of cyclophosphamide (CY)-treated mice, in which the results were displayed as means±S.D. (n=4 to 6 for each group), and data were analyzed by Student's t-test (test group vs. control, *: P < 0.05 and **: P < 0.01);

Please amend the paragraph beginning at page 17, line 13, as follows:

To prepare an injection, a pH regulator, a buffer, a stabilizer, an isotonicity and the like may be admixed with an extract product from the root of *Polygonum multiflorum* Thunb. according to this invention. The resultant mixture can then be formed into a subcutaneous, intramuscular or intravenous injection by a method known per se in the art. Examples of the pH regulator and buffer include sodium citrate, sodium acetate, and sodium phosphate. Illustrative of the stabilizer include sodium pyrosulfite, ~~EDTA~~ ethylenediamine tetraacetic acid (EDTA), thioglycolic acid, and thiolactic acid. Examples of the isotonicity include sodium chloride and glucose.

Please amend the paragraph starting at page 18, line 26, as follows:

The reverse-phase HPLC elution profile of PoMuM was detected under 312 nm (~~red~~ middle line (20)), 254 nm (~~blue~~ upper line (10)) and 280 nm (~~green~~ lower line (30)), respectively, and the obtained results are shown in Figure 1.

Please amend the paragraph starting at page 21, line 5, as follows:

Briefly, the mouse liver was first perfused *in situ* through the portal vein with a Ca²⁺ - free Hanks' solution containing 5 mM ~~EGTA~~ ethylene glycol-bis(β-aminoethyl ether)-N,N,N',N'-

tetraacetic acid (EGTA) at 37°C for 10 minutes, followed by perfusion with a 0.05% collagenase solution for 10 minutes at pH 7.4 at 37°C. The perfused liver was excised and dispersed in a cold Hanks' solution, and the resultant liver cell suspension was filtered through a double layer of gauze and divided into two fractions.

Please amend the paragraph beginning at page 21, line 20, as follows:

The mouse hepatocytes as prepared above were suspended at a density of 5×10^3 viable cells/150 μ L ~~DMEM~~ Dulbecco modified Eagle medium (DMEM) culture medium containing 30 μ g/ml L-proline, 10^{-7} M Dexamethasone and 5 μ g/ml insulin supplemented with 10% ~~FCS~~ fetal calf serum (FCS) and were then placed into each well of 1% gelatin-coated 96-well culture plates. After incubation at 37°C under 95% air plus 5% CO₂ for 2-3 hours, a monolayer of hepatocytes was formed and adhered on the bottom wall of each well. The medium and dead hepatocytes in each well was removed, and nonparenchymal cells in 10% FCS in DMEM at a density of 5×10^4 cells per well were added. After overnight incubation, the cells in each well were washed twice with PBS and maintained with 180 μ l serum-free DMEM medium supplemented with 1 mg/ml galactose, 30 μ g/ml L-proline, 0.5 μ g/ml insulin, 10^{-7} M dexamethasone and 10 ng/ml EGF. The culture plates were placed in a 37°C humidified incubator with 5% CO₂/95% air atmosphere and incubated for 1 hour before conducting the following assays.

Please amend the paragraph beginning at page 29, line 3, as follows:

Subsequently, MTT assay was performed. Each well of the culture plates was added with a MTT solution (5mg/ml dissolved in 1X PBS) to a final concentration of 1 mg/ml. Four hours later, each well of the culture plates was added with a MTT lysis buffer (20% ~~SDS~~ sodium

dodecyl sulfate (SDS) in 50% ~~DMF~~ dimethylformamide (DMF)/ 50% H₂O) in an amount of 150 µl/well. The culture plates were allowed to stand for 14 hours and then subjected to absorbance detection using a microplate reader at O.D. 550 nm-O.D. 690 nm. Each experiment was conducted in duplicate, and n=6 wells for each group. The obtained experimental data were analyzed by Student's *t*-test.

Please amend the paragraph beginning at page 32, line 26, as follows:

Under sterile condition, C57/BL6j (male, 15-20g, 4-6 weeks old) were sacrificed, and their femoral bones were injected with ~~α-MEM~~ alpha-minimal essential medium (α- MEM) culture medium (Gibco, NY, USA) so as to flush out the bone marrow cells. The collected cells in α- MEM culture medium were then filtered through a sterile No. 53 nylon mesh so as to obtain a single cell suspension.

Please amend the paragraph beginning at page 37, line 14, as follows:

The mouse bone marrow cells (1.5x10⁵ nucleated cells/well) prepared according to the procedures set forth in Procedure (I) of Example 5 were placed into U-shaped 96-well culture plates and incubated in α-MEM supplemented with 1% ~~BSA~~ bovine serum albumin (BSA), 7.5 µM 2-mecaptoethanol, 1.4 mM L-glutamine, 10 µM FeCl₃ and EPO (at a final concentration of 50 mU/well). After incubation at 37°C for 24 hrs, extracts of Polygonum multiflorum Thunb. root with different concentrations (0.01 µg/ml, 0.1 µg/ml, 1 µg/ml, 10 µg/ml, 100 µg/ml and 1000 µg/ml) were added into the wells of the culture plates, respectively. The positive control group was treated with EPO at a concentration of 500 mU/well, and the negative control group was treated with EPO at a concentration of 50 mU/well. The culture plates were then incubated in a 37°C incubator containing 5% CO₂ for 96 hrs. Subsequently, a colorimetric assay for

hemoglobin (Rosenthal, A. et al. (1985), *Experimental Hematology*, 13: 174184; Worthington, R. E., et al. (1987), *Experimental Hematology*, 15: 85-92) was performed to determine the extent of proliferation of mouse bone marrow hematopoietic progenitor cells.

Please amend the paragraph beginning at page 46, line 25, as follows:

On Day 5, the mice in each group were sacrificed, and total mRNAs were extracted from the bone marrow cells collected therefrom. 5 µg of the extracted total mRNAs and 2.5 µg of oligo dT were mixed at 70°C for 10 minutes and then placed at room temperature for 10 minutes. Subsequently, the mixture was added with 4 µl of 10 mM ~~dNTP~~ deoxyribonucleotide triphosphate (dNTP), 0.5 µl of rRNasin, and 1 µl AMV (Avian Myeloblastosis virus) reverse transcriptase (10 units) as well as the buffer thereof, so as to form a final reaction volume of 26.5 µl. The reverse transcription reaction was carried out by heating the mixture at 42°C for 60 min, followed by heating at 90°C for 5 min. Thereafter, 2.5 µl of cDNA formed therefrom was added with 0.5 µl 10 mM dNTP, the forward and reverse primers (for each primer, 1 µl in a concentration of 1 µg/µl) of a target cytokine (~~IL-1β~~ Interleukin-1β (IL-1β), ~~IL-6~~ Interleukin-6 (IL-6), ~~G-CSF~~ Granulocyte Colony-Stimulating Factor (G-CSF), GM-CSF or ~~SCF~~ Stem Cell Factor (SCF)), and 0.5 µl polymerase (2 units) as well as the buffer thereof, so as to form a final reaction volume of 25 µl. The polymerase chain reaction (PCR) was performed in a DNA thermal cycler (Perkin-Elmer-Cetus) for 35 cycles, each cycle consisting of: denaturation at 94°C for 45 sec., annealing at an appropriate temperature for 45 sec., and extension at 72°C for 1 min. The resultant PCR products were subjected to electrophoresis on 2% agarose gels and visualized by ethidium bromide staining.